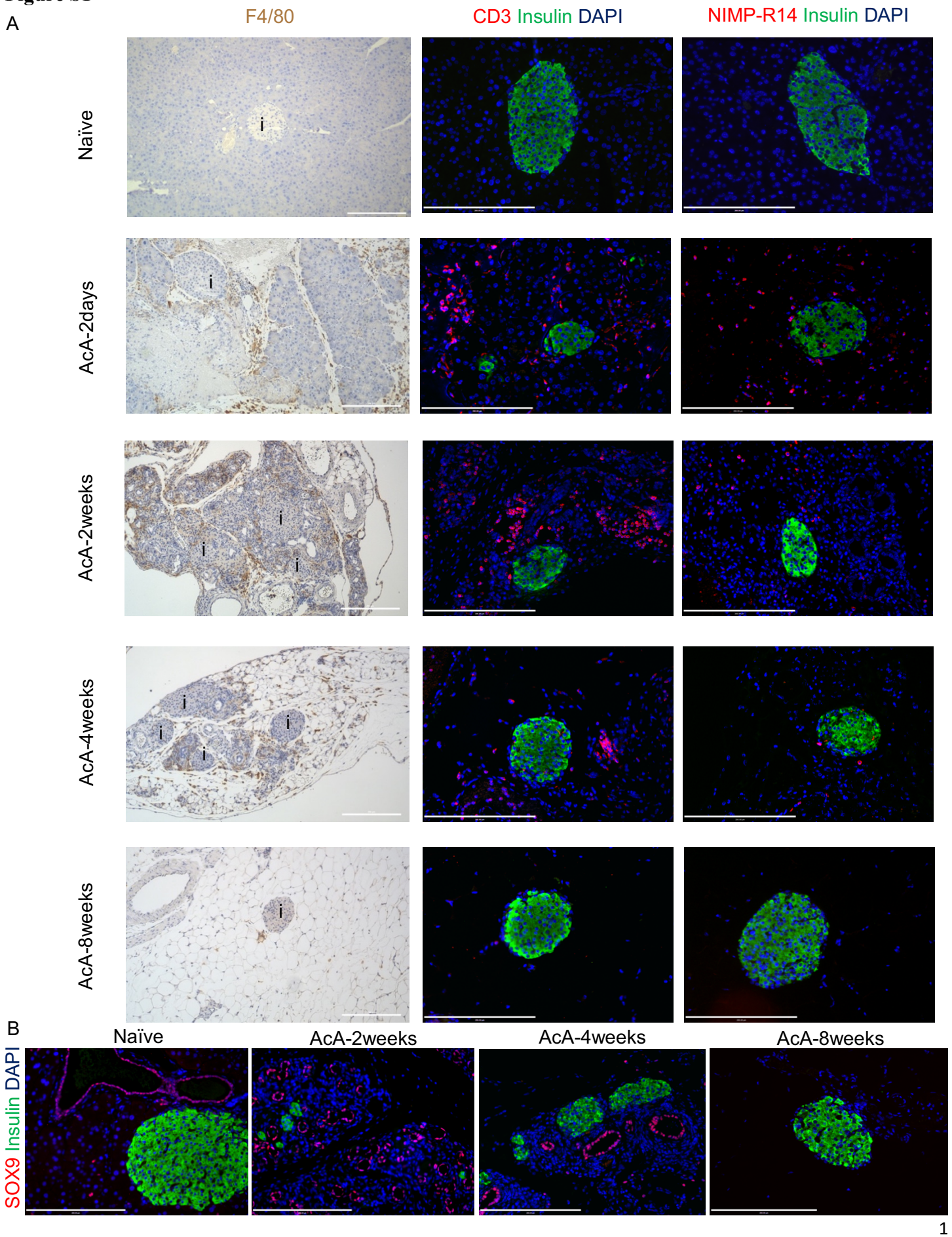
Figure S1


A
F4/80
CD3 Insulin DAPI
NIMP-R14 Insulin DAPI
Naïve
AcA-2days
AcA-2weeks
AcA-4weeks
AcA-8weeks
i
B
Naïve
AcA-2weeks
AcA-4weeks
AcA-8weeks
SOX9 Insulin DAPI

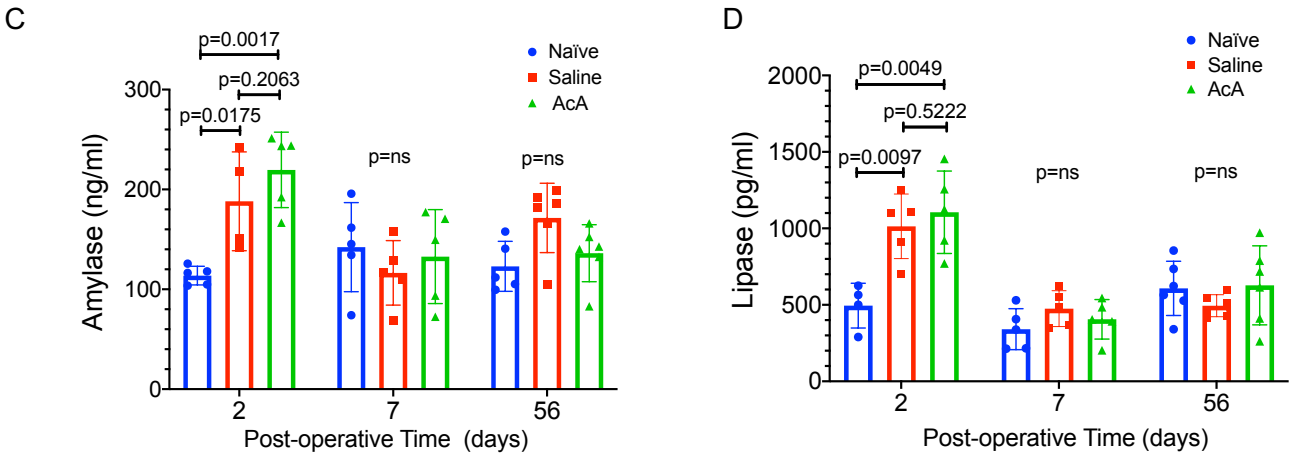


Figure S1: Time course of inflammatory and ductal changes in the exocrine pancreas following chemical pancreatectomy.

A: Immunostaining with F4/80 (macrophage marker), CD3 (lymphocyte marker), NIMP-R14 (neutrophil marker), and insulin in control and AcA-infused pancreata showed inflammatory infiltration in the exocrine pancreas that spared the islets at 2-days, with a peak at 2-weeks, followed by regression at 4-weeks and resolution at 8-weeks.

B: Immunostaining for SOX-9 (duct-cell marker) in control and AcA-infused pancreata showed the presence of ducts/duct-like structures at 2-weeks and 4-weeks. While at 8-weeks, immunostaining was negative for SOX-9, suggesting involution of the ducts.

C: Measurement of serum amylase at serial time-points post-operatively; at 2-days, serum amylase was elevated in AcA and saline-treated mice compared to naïve mice, (n= 5/naïve, 4/saline, 5/AcA), one-way ANOVA $F_{(2,11)}$=12.04; p=0.0017). At 7-days, there was no significant difference between the 3 groups (n=5/group), (one-way ANOVA $F_{(2,12)}$=0.4875; p=0.6258). At 8-weeks, there was no significant difference between the 3 groups (n=5/naïve, 6/saline, 6/AcA), (one-way ANOVA $F_{(2,13)}$=2.704; p=0.1043).

D: Measurement of serum lipase at serial time-points post-operatively; at 2-days serum lipase was elevated in AcA and saline-treated mice compared to naïve mice (one-way ANOVA $F_{(2,11)}$=9.627; p=0.0038), (n=4/naïve, 5/saline, 5/AcA). At 7-days, there was no significant difference between the 3 groups (n=5/group), (one-way ANOVA $F_{(2,12)}$=1.403; p=0.2835) At 8-weeks, there was no significant difference between the 3 groups (n=6/group), (one-way ANOVA $F_{(2,15)}$=0.903; p=0.4263)

Illustrative results from 5 animals/time-point are shown. Scalebar=200 µm.

Data are presented as mean±S.D. Multiple comparisons were performed by Holm-Sidak's test. NS=non-significant.

Figure S2

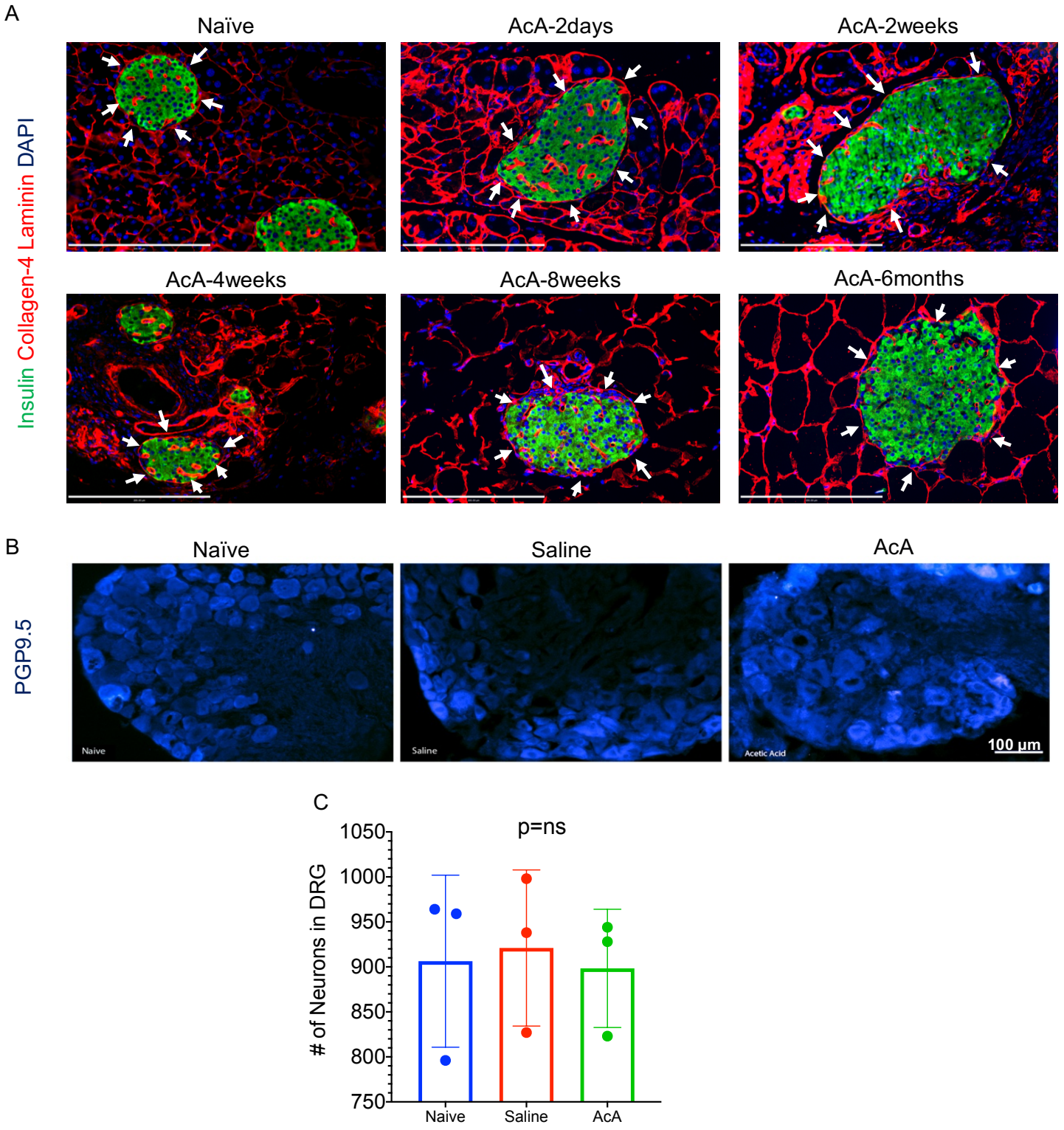


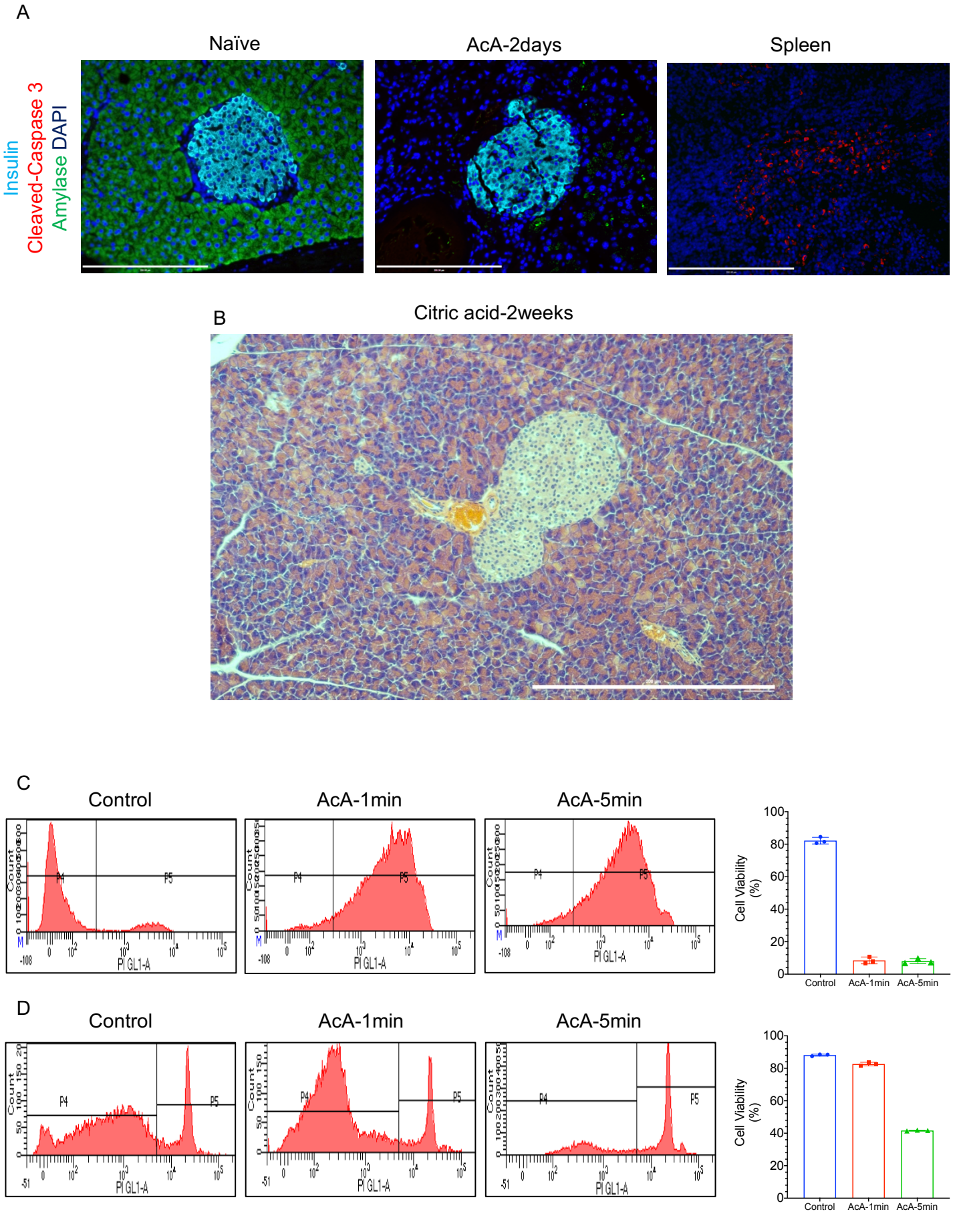
Figure S2: Effect of chemical pancreatectomy on the islet capsule and pancreatic sensory neurons

A: Immunostaining for laminin and collagen IV (basement membrane markers), and insulin in the pancreas, showed an intact islet capsule (arrows) in AcA-treated mice at all timepoints.

B,C: Immunostaining for PGP9.5 in the T12 DRG, which were harvested 8-weeks post-surgery **(B)**. Counting of neurons in the T12 DRG showed no significant difference in the number of neurons among naïve, saline-treated, and AcA-treated mice (n=3/group), (one-way ANOVA $F_{(2,6)}$=0.05668; p=0.9454) **(C)**.

Data are presented as mean±S.D. Illustrative histology results from 3 animals/time-point are shown. Scalebar=200 μm unless otherwise specified. NS=non-significant.

Figure S3


A
Naïve
AcA-2days
Spleen
Insulin
Cleaved-Caspase 3
Amylase DAPI
B
Citric acid-2weeks
C
Control
AcA-1min
AcA-5min
Count
P4
P5
PI GL1-A
Cell Viability (%)
Control
AcA-1min
AcA-5min
D
Control
AcA-1min
AcA-5min
Count
P4
P5
PI GL1-A
Cell Viability (%)
Control
AcA-1min
AcA-5min

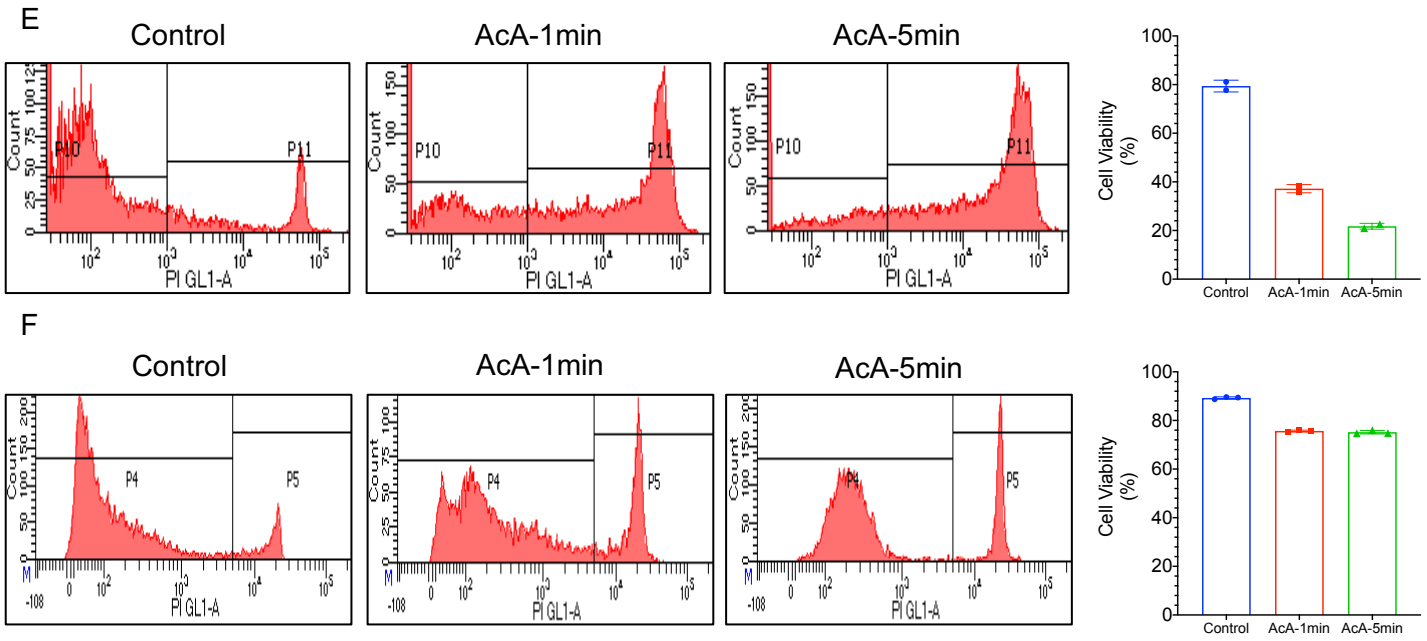


Figure S3: Mode of cell death and acinar cells susceptibility to cell injury after AcA treatment

A: Immunostaining for cleaved-caspase-3 (marker of apoptosis), insulin and amylase 2-days post-AcA-infusion showed marked decline in amylase staining in AcA-infused pancreata with negative caspase-3 staining, indicating that apoptosis is likely not the cause of acinar cell death. Spleen was used as a positive control for caspase-3.

B: Pancreas histology 2-weeks post-citric acid infusion revealed normal histology indicating that pH is not the determining factor of cell death after AcA-infusion.

C-D: FACS quantification of PI^+ cells (dead cells) in acinar **(C)** and islet **(D)** cells 1-hour after harvesting. The panel shows cells exposed to PBS (control) and after exposure to AcA (1 & 5 min) and a representative graph for the FACS data (n=3/group).

E-F: FACS quantification of PI^+ cells in islets 24 hours after harvesting. The panel shows islet cells exposed to PBS (control) and islet cells after exposure to AcA (1 & 5 min). Islets were dissociated before the AcA exposure (n=2) **(E),** while islets were intact during the exposure to AcA (n=3), followed by dissociation for FACS **(F)**. A representative graph for the FACS data is shown.

Illustrative histology results from 3 animals are shown. Scalebar=200 µm. Data are presented as mean±S.D.

Figure S4

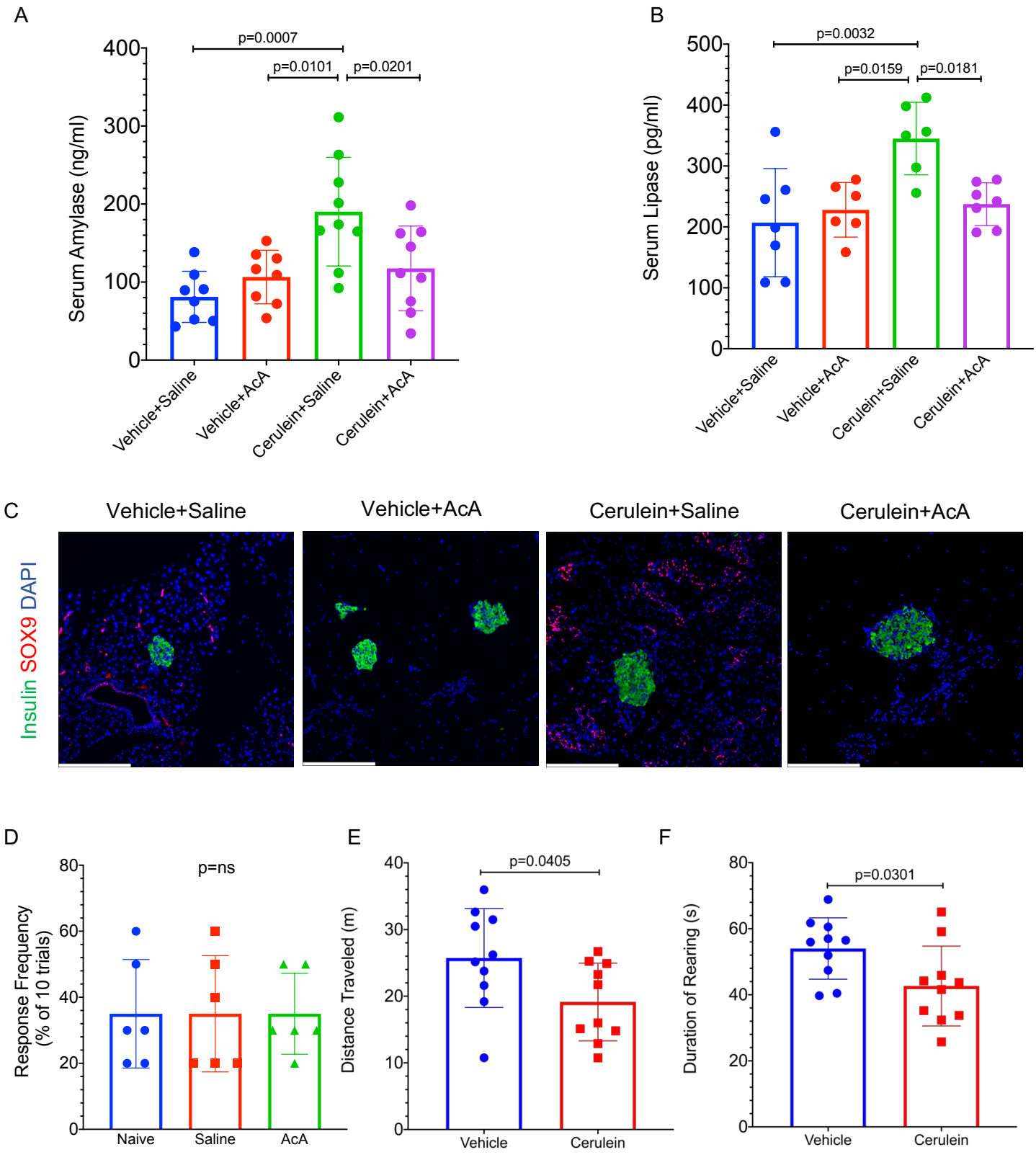

A
Serum Amylase (ng/ml)
p=0.0007
p=0.0101
p=0.0201
Vehicle+Saline
Vehicle+AcA
Cerulein+Saline
Cerulein+AcA
B
Serum Lipase (pg/ml)
p=0.0032
p=0.0159
p=0.0181
C
Vehicle+Saline
Vehicle+AcA
Cerulein+Saline
Cerulein+AcA
Insulin SOX9 DAPI
D
Response Frequency (% of 10 trials)
p=ns
Naive
Saline
AcA
E
Distance Traveled (m)
p=0.0405
Vehicle
Cerulein
F
Duration of Rearing (s)
p=0.0301
Vehicle
Cerulein

Figure S4: Chemical pancreatectomy resolves inflammation, ablates ducts and ameliorates pain-like behavior in cerulein-induced CP

A,B: Serum amylase **(A)** and lipase **(B)** concentrations were significantly higher in Cerulein+saline compared to the other 3 subgroups. In **(A)**, cerulein+saline (n=9), cerulein+AcA (n=9), vehicle+saline (n=8), and vehicle+AcA (n=8), (one-way ANOVA, $F_{(3,30)}$=7.298; p=0.0008). In (**B**), cerulein+saline (n=6), cerulein+AcA (n=7), vehicle+saline (n=7), and vehicle+AcA (n=6), (one-way ANOVA, $F_{(3,22)}$=6.315; p=0.003).

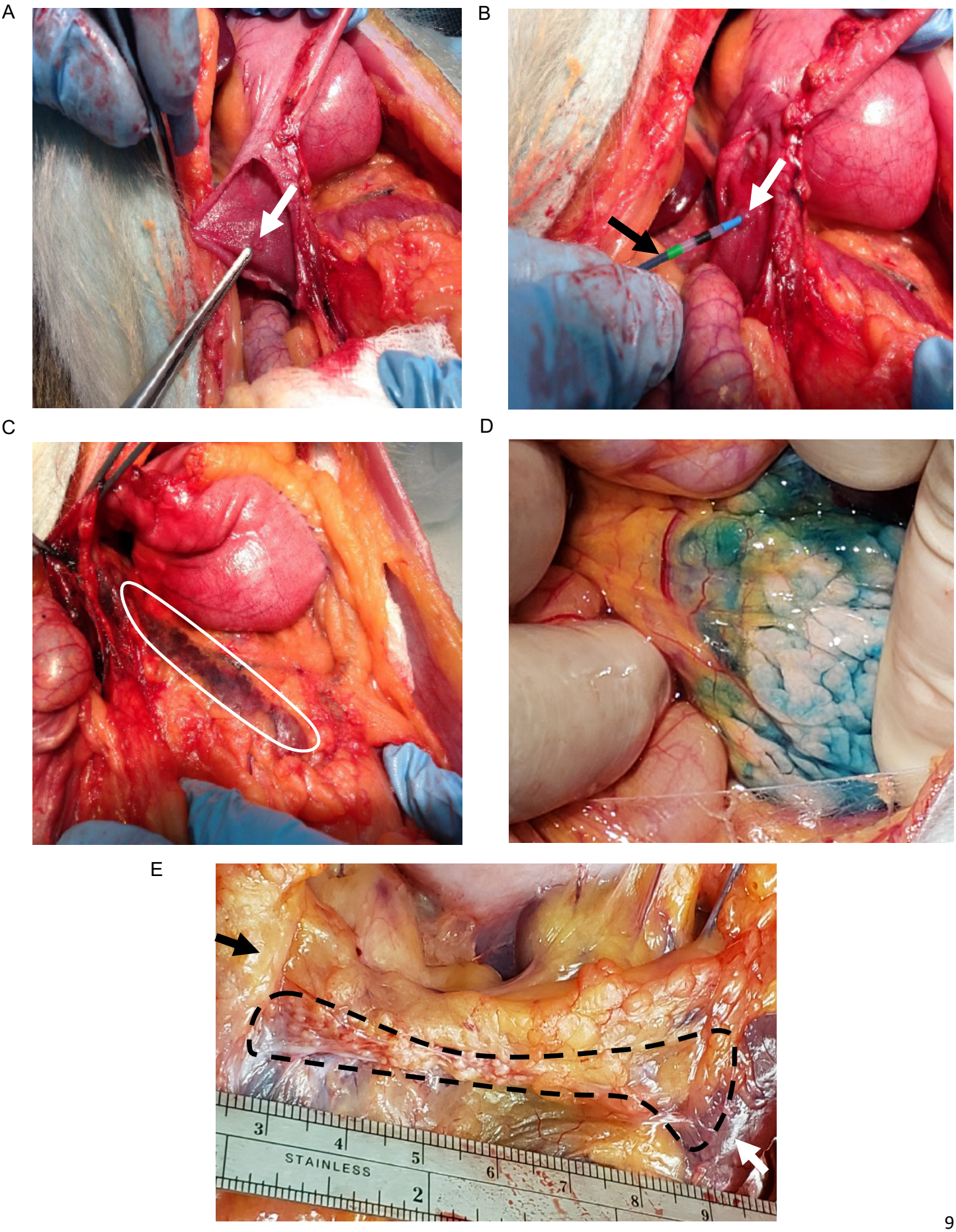
C: Immunostaining for SOX9 showed absence of ducts only in the AcA-treated groups indicating involution of the ducts.

D: Baseline abdominal Von-Frey monofilament assay was performed 8-weeks post-AcA- or saline-infusion in otherwise unperturbed mice to detect abdominal mechanical sensitivity as a measurement for pain due to AcA or saline treatment alone. There were no differences among the three groups (naïve, saline and AcA). Response frequency as a percentage of 10 trials was measured, n=6/group, (one-way ANOVA, $F_{(2,15)}$=1; p=0.3911).

E,F: Open field test to assess pain-like behavior was performed following 8 weeks of IP cerulein or vehicle as a model of CP. Distance traveled (unpaired t-test, $t_{(18)}$=2.208; p=0.0405), **(E)** and rearing duration (unpaired t-test, $t_{(18)}$=2.354; p=0.0301), **(F)** were measured showing pain-like behavior in the cerulein-induced CP, n=10/group.

Illustrative histology results from 4 animals/time-point are shown. Scalebar=200 μm. Data are presented as mean±S.D. Multiple comparisons were performed by Holm-Sidak's test. NS=non-significant. In **A**, and **B**, only significant p-values are depicted.

Figure S5



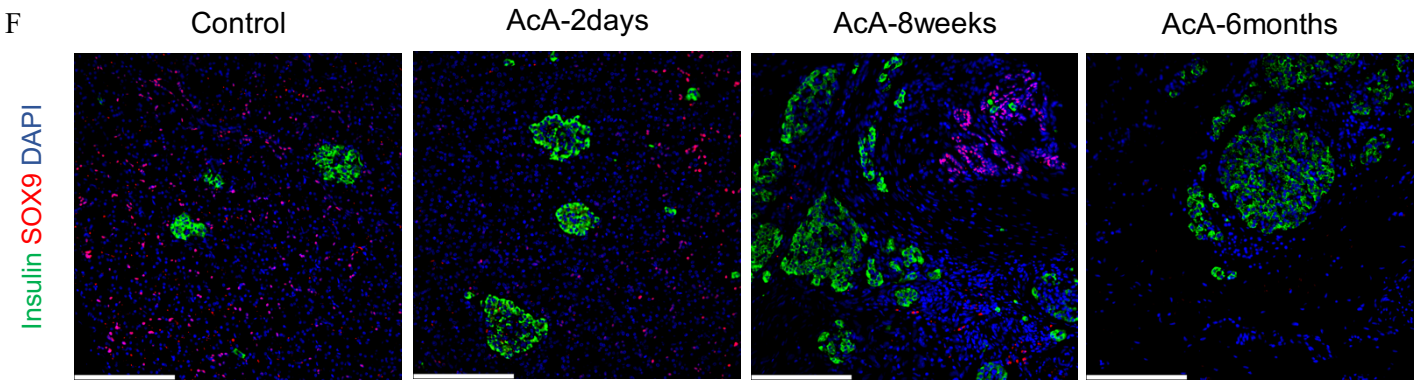


Figure S5: Chemical pancreatectomy in NHPs

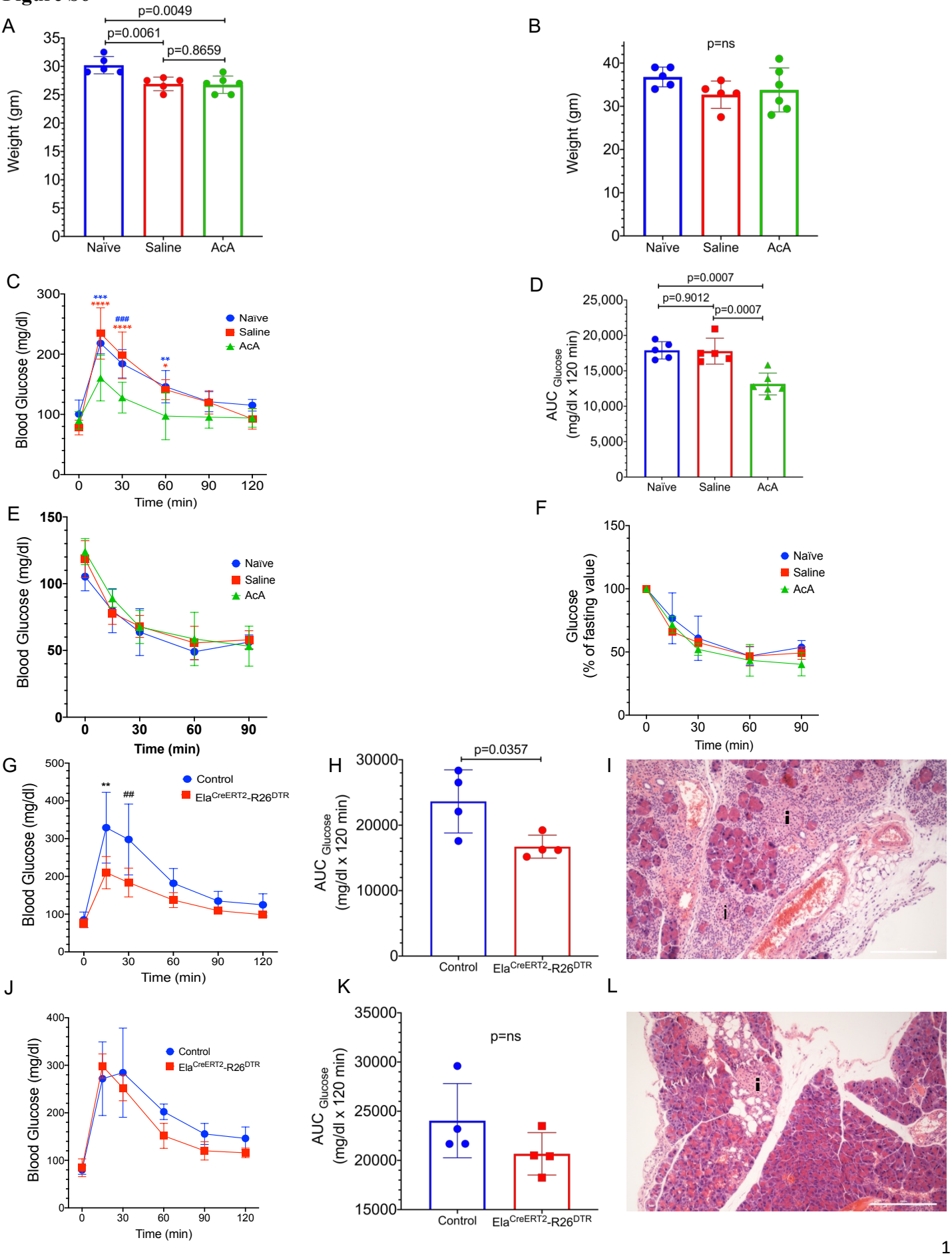
A-C: Necropsy of a normal Cynomolgus macaque monkey. **(A)** Duodenotomy shows the normal location of the opening of the pancreaticobiliary duct (ampulla of Vater) denoted by the white arrow, identical to human anatomy. **(B)** The ERCP catheter (black arrow) is inserted through the ampulla (white arrow) and specifically into the main pancreatic duct. **(C)** Dye injection through the ERCP catheter into the pancreatic duct demonstrates complete perfusion of the pancreas (outlined dark-stained tissue).

D: Intraoperative picture of an NHP pancreas during the infusion of AcA and methylene blue, showing complete perfusion of the pancreas.

E: Necropsy of a Cynomolgus macaque at 8-weeks post-AcA-infusion revealed a marked loss of the normal-appearing exocrine tissue with pancreas currently at approximately 10% of its former size (see Fig.8 for the histologic appearance) as shown between the duodenum (black arrow) and the spleen (white arrow).

F: Immunostaining for SOX9 showed presence of ducts at 2-days and 8-weeks post-AcA-infusion. However, at 6-months, the SOX9 immunostaining was negative, indicating absence of ducts. Illustrative results from 2 animals/time-point are shown (for 6-months timepoint, n=1). Scalebar=200 µm.

Figure S6


A
p=0.0049
p=0.0061
p=0.8659
Weight (gm)
Naïve
Saline
AcA
B
p=ns
Weight (gm)
Naïve
Saline
AcA
C
Blood Glucose (mg/dl)
Naïve
Saline
AcA
Time (min)
D
p=0.0007
p=0.9012
p=0.0007
AUC Glucose (mg/dl x 120 min)
Naïve
Saline
AcA
E
Blood Glucose (mg/dl)
Naïve
Saline
AcA
Time (min)
F
Glucose (% of fasting value)
Naïve
Saline
AcA
Time (min)
G
Blood Glucose (mg/dl)
Control
ElaCreERT2-R26DTR
Time (min)
H
p=0.0357
AUC Glucose (mg/dl x 120 min)
Control
ElaCreERT2-R26DTR
I
J
Blood Glucose (mg/dl)
Control
ElaCreERT2-R26DTR
Time (min)
K
p=ns
AUC Glucose (mg/dl x 120 min)
Control
ElaCreERT2-R26DTR
L

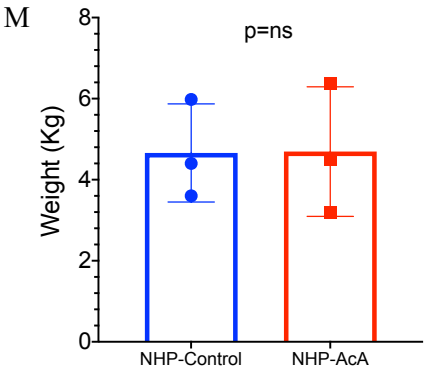


Figure S6: Effect of chemical pancreatectomy on endocrine function

A, B: Two-weeks post-surgery, the body weight of AcA-treated mice (n=6) and saline-treated mice (n=5) was significantly lower than naïve mice (n=5) (one-way ANOVA $F_{(2,13)}$=9.523; p=0.0028) **(A)**. Eight-weeks post-surgery, body weight was not significantly different between the 3 groups (n=5/naïve, 5/saline, 6/AcA) (one-way ANOVA $F_{(2,13)}$=1.551; p=0.2488) **(B)**.

C,D: IPGTT at 2-weeks **(C)** showed significant improvement in glucose tolerance in the AcA group (n=6), compared to controls (n=5/group). In **(C)**, *p=0.0105,**p=0.006, ***p=0.0006,****p<0.0001, ###p=0.0009, (two-way repeated-measures ANOVA $F_{(10,65)}$=2.951; p=0.0041). Analysis of AUC **(D)**, (one-way ANOVA $F_{(2,13)}$=17.13; p=0.0002).

E,F: ITT at 2-weeks (n= 4/naïve, 5/saline, and 5/AcA) **(E)**. **(F)** represents percent change in glucose levels to its own fasting level (two-way repeated-measures ANOVA $F_{(8,40)}$=0.8238; p=0.5866).

G-I: IPGTT performed 2-days post-DT **(G)**, showed improved glucose tolerance in $Ela^{CreERT2}$-$R26^{DTR}$ compared to their littermate controls (n=4/group). In **(G)**, **p=0.0044, ##p=0.0057, (two-way repeated-measures ANOVA, $F_{(5,30)}$=3.15; p=0.0211). Analysis of AUC **(H)**, (unpaired t-test, $t_{(6)}$=2.698; p=0.0357). Pancreatic histology of $Ela^{CreERT2}$-$R26^{DTR}$ mice 2 days **(I).** Pancreatic histology of $Ela^{CreERT2}$-$R26^{DTR}$ mice 2 days post-DT **(I),** (i) denotes islets.

J-L: IPGTT performed 3-weeks post-DT **(J)**, showed no significant difference between the $Ela^{CreERT2}$-$R26^{DTR}$ and their littermate controls (n=4/group), (two-way repeated-measures ANOVA $F_{(5,30)}$=1.428; p=0.243). Analysis of AUC **(K)**, (unpaired t-test, $t_{(6)}$=1.552; p=0.1717). Pancreatic histology of $Ela^{CreERT2}$-$R26^{DTR}$ mice 3 weeks post-DT **(L),** (i) denotes islets.

M: During the IVGTT, the body weight of AcA-treated NHPs was not different from controls (n=3/group), (unpaired t-test, $t_{(4)}$=0.02879; p=0.9784).

Illustrative histology results from 3 animals are shown. Scalebar=200 μm. Data are presented as mean±S.D. Multiple comparisons were performed by Holm-Sidak's test. NS=non-significant.

Table S1: Comprehensive metabolic profile for NHPs at baseline and 2 days post-surgery

	Baseline	2 Days Post-surgery	*p*-value
Sodium (Na)	145.25±2.06	147.5±1.73	0.14
Potassium (K)	4.85±1.04	4±0.35	0.09
Chloride (Cl)	111.75±3.59	115.5±4.44	0.36
Calcium (Ca)	8.33±0.38	7.9±0.55	0.20
Glucose	84.5±61.04	81.5±34.14	0.95
Albumin	2.93±0.31	2.48±0.53	0.35
Blood Urea Nitrogen (BUN)	13.75±2.36	15.25±4.11	0.64
Bicarbonate	26±3.46	23.75±4.92	0.50
Alanine Aminotransferase (ALT)	30.25±6.85	45.75±27.15	0.32
Aspartate Aminotransferase (AST)	32±8.76	51.75±28.12	0.36
Creatinine	0.7±0.25	0.51±0.26	0.48
Total Protein	6.25±0.52	6±0.87	0.71
Total Bilirubin	0.2±0.08	0.185±0.1	0.84
Alkaline Phosphatase	388.5±233.43	295±137.93	0.43

N = 4 NHPs. Statistics were performed using paired t-test.

Table S2. Primary antibodies used in IHC

Name of Antibody	Manufacturer / Catalog Number	Source Species	Dilution Used
Insulin	Abcam/ab195956	Guinea pig	1/500
Glucagon	Abcam/ab92517	Rabbit	1/1000
Glucagon	Abcam/ab10988	Mouse	1/1000
PGP9.5	Fischer Scientific /PA1-10024	Rabbit	1/1000
F4/80	Abcam/ab6640	Rat	1/200
Amylase	Sigma/A8273	Rabbit	1/300
CD31	Abcam/ab222783	Rabbit	1/50
CD3	DAKO/a045229-2	Rabbit	1/150
SOX9	Abcam/ab185966	Rabbit	1/1000
Anti-Neutrophils (NIMP-R14)	Abcam/ab53457	Rat	1/200
Caspase-3	Abcam/ab208161	Mouse	1/200
Collagen 4	Abcam/ab19808	Rabbit	1/200
Laminin	Abcam/ab11575	Rabbit	1/200

Table S3. RT-PCR Primers

Gene	Accession Number	Forward (5'→3')	Reverse (3'→5')
calca	NM_007587.2	AAG CCA GAA CCA TGC TGT CAT	ACC AAT GTG GGC TCT GAA GC
gapdh	NC_000072.6	ATG TGT CCG TCG TGG ATC TGA	ATG CCT GCT TCA CCA CCT TCT T